2003 MAFMA Final Report

Project Title: Control of *Listeria monocytogens* in Ready-to-Eat Meat Products Using Chemical Antimicrobial (Buffered Sodium Citrate and Buffered Sodium Cintrate Supplemented with Sodium Diacetate) and Staurated Steam.

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1. Objective Summary

The objectives of the proposed study were (1) Determine potential synergistic lethal effects of organic acid salt treatments of products, ***as part of the product formulation***, prior to in-package thermal applications for *L. monocytogenes* control and (2) Evaluate repair and potential outgrowth of *L. monocytogenes* inoculated at low levels during storage subsequent to steam-based in-package pasteurization of RTE meat products.

2. Objective Accomplishments

L. monocytogenes contamination has become a major concern for the ready-to-eat meat industry. Post-processing contamination of ready-to-eat meats at very low initial populations can occur, *L. monocytogenes* can grow to potentially hazardous levels during refrigerated storage and result in foodborne listeriosis from. Technologies to minimize the risk of listeriosis should be applied to control growth of *L. monocytogenes* during refrigerated storage.

The inhibitory effects of organic acids salts (lactate/diacetate and citrate/diacetate combination antimicrobials) and post-process pasteurization using saturated steam to control *L. monocytogenes* growth on frankfurters and sliced hams was evaluated along with microbiological shelf-life refrigerated storage.

Frankfurters and sliced hams were surface inoculated with a five-strain mixture of *L. monocytogenes* (ca. 10^5 CFU/mL), vacuum packaged, surface pasteurized using saturated steam at 96.1ºC. The products were cooled, stored under refrigeration and sampled for *L. monocytogenes* populations, microbiological quality, pH and a_w at 15 day intervals.

Post-process pasteurization (96.1°C for 2 min 10 s) reduced *L. monocytogenes* populations by 0.77 log CFU/cm^2 from initial populations of ca. 1.78 log CFU/cm^2 on frankfurters. Subsequent growth resulted in final populations of ca. 6.00 log CFU/cm^2 in control and in product containing lactate/diacetate combination of antimicrobials.

Incorporation of citrate/diacetate combination antimicrobials (1.0 or 1.3%) inhibited *L. monocytogenes* growth on frankfurters during refrigerated storage.

Post-process pasteurization of hams resulted in ca. 0.4 log CFU/cm^2 ($p>0.05$) reduction in initial *L. monocytogenes* populations (ca. 1.15 log CFU/cm^2). *L. monocytogenes* was able to grow to ca. 5.00 log CFU/cm^2 on control hams within 30 and 45 days of storage at 10 and 4°C, respectively. Incorporation of antimicrobials (both lactate/diacetate and citrate/dicetate combinations) resulted in inhibition of *L. monocytogenes* growth on hams during storage at both temperatures.

Use of antimicrobial agents as part of the formulation in addition to surface heat treatment provides a good alternative to reduce the risk of listeriosis from ready-to-eat meat products.

3. Unexpected findings, if any
   None observed.

4. Practical impacts of research efforts.

   a. Short Term Impacts: Subsequent to this research, several meat and poultry processors have incorporated the buffered sodium citrate with diacetate (combination) as part of their strategy to control *Listeria monocytogenes* in their ready-to-eat meat and poultry products. This research has been the basis for further validation of antimicrobial activity of buffered sodium citrate and sodium diacetate in other RTE meat and poultry products.

   b. Long Term Impacts: The USDA Food Safety and Inspection Service in collaboration with US Food and Drug Association and Centers for Disease Control and Prevention has conducted a risk assessment and reported that the RTE meat and poultry products present a significant risk of foodborne illness due to *Listeria monocytogenes*. Subsequent to the publication of this risk assessment, USDA-FSIS published a final rule "Control of *Listeria monocytogenes* in Ready-to-Eat Meat and Poultry Products" requiring the RTE meat and poultry processors to incorporate *Listeria monocytogenes* control strategies into their processing systems. These include incorporation of antimicrobial agents or antimicrobial process (subsequent to cooking) to control the growth of *L. monocytogenes* or reduce their populations. Alternatively, the processors could incorporate both these strategies, where the populations of this pathogen would be reduced by the antimicrobial/lethality process and the growth during refrigerated storage would be controlled by use of the antimicrobial agents or process (such as freezing) to provide an additional measure of safety.

This project provided the RTE meat industry with a solution to the *Listeria* problem, by exploiting the lethal effect of thermal treatment and bacteriostatic effect of organic acid salts, preventing *Listeria* outgrowth during the shelf life of the product. This will assure that the risk of *Listeria monocytogenes* is reduced even at the end of the

product's shelf life, greatly reducing the risk of foodborne listeriosis along with associated benefit to the meat industry by reducing the risk of meat product recalls.

5. If you are also making reports to other funding agencies in the course of this research work, please include a copy of that report.

6. a. Publications resulting from this research.

A research paper is being prepared from this research and will be submitted to a peer reviewed journal for publication. The suggested wording will be included to attribute support of the project to the CSREES special research grant.

b. If any patents (pending or granted) resulted from the research, please include the patent information.

Control of *Listeria monocytogenes* in Ready-to-Eat Meat Products Using Chemical Antimicrobials (Buffered Sodium Citrate and Buffered Sodium Citrate Supplemented with Sodium Diacetate) and Saturated Steam.

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ABSTRACT

Control of *L. monocytogenes* using a combination of a post-lethality thermal pasteurization using saturated steam and the incorporation of salts of organic acids as part of the formulation of ready-to-eat frankfurters was evaluated. Frankfurters were formulated to contain no antimicrobials (control), a combination of sodium lactate and sodium diacetate (2.3 and 0.17%, respectively; lactate/diacetate combination) at 2.5% in the finished product, or a combination of buffered sodium citrate and sodium diacetate (citrate/diacetate combination) at either 1.0 or 1.3% in the finished product. A five strain cocktail of *L. monocytogenes* was surface inoculated on the frankfurters and vacuum packaged. For treatments requiring heat treatment, frankfurters were exposed to saturated steam at 96.1ºC for 5 min and 10s, chilled and subsequently stored at 4ºC for up to 120 days.

Surface *L. monocytogenes* populations of ca. 1.78 log CFU/cm^2 were attained after inoculation of frankfurters. Application of a post-packaging thermal pasteurization (96.1°C for 2 min 10 s) reduced *L. monocytogenes* populations by 0.77 log CFU/cm^2 ($p>0.05$), while subsequent growth resulted in final populations of ca. 6.00 log CFU/cm^2 in control (product that did not contain any antimicrobials) and in product containing lactate/diacetate combination of antimicrobials. Incorporation of citrate/diacetate combination antimicrobials (1.0%) inhibited *L. monocytogenes* growth on frankfurters with maximal populations of 3.91 and 2.16 log CFU/cm^2 during storage. Increasing citrate/diacetate combination antimicrobial concentration to 1.3% resulted in greater inhibition of *L. monocytogenes*, with final populations of 2.99 log CFU/cm^2 on day 120. Use of antimicrobial agents as part of the formulation in addition to surface heat treatment provides a good alternative to reduce the risk of listeriosis from ready-to-eat meat products.

INTRODUCTION

The Food Safety and Inspection Service established an interim final rule for control of *L. monocytogenes* in RTE meat and poultry products. Food processors are required to comply with one of three alternatives to prevent product adulteration with the pathogen. Alternative 1 requires the application of both a post-lethality treatment (may be an antimicrobial agent) to reduce or eliminate *L. monocytogenes* and antimicrobial agent or process to limit or suppress growth of the pathogen. Alternative 2 requires employing either a post-lethality treatment or growth inhibitor. Alternative 3 relies on controlling pathogen with sanitation measures only. Establishments that choose this option, however, are required to conduct testing of food contact surfaces to confirm the efficacy of sanitation procedures in the post-lethality processing environment and to develop product-holding procedures when positive tests are obtained (7).

While extensive research has been conducted on the effectiveness of sodium or potassium salts or organic acids such as lactic acid in combination with sodium diacetate for controlling *Listeria monocytogenes* in RTE meat products, other organic acid salts such as citric acid salts have not been evaluated. Further, sodium citrate and citric acid (Buffered Sodium Citrate, Ional™; 6) are Generally Recognized as Safe (GRAS) ingredients and have been shown to inhibit growth of pathogens in meat products.

Buffered sodium citrate (Ional™) and buffered sodium citrate in combination with sodium diacetate (Ional Plus™) have been shown to inhibit germination and outgrowth of *Clostridium perfringens*, a pathogen used as performance standard during chilling of meat products (5). Several of the meat processors are presently incorporating this antimicrobial ingredient as a secondary inhibitor for control of *C. perfringens*.

Saturated steam provides unique advantages over other methods of heating in that the huge latent heat of evaporation/condensation of water makes the surface heating rate uniquely high compared to hot water pasteurization systems. A rapid product surface temperature rise to lethal temperatures (>160 °F) can be obtained within 15 sec with minimal internal product temperature rise, and a rapid chilling of the product surfaces to 45 °F within 5 sec using a chilled water or cryogenic chilling system can be achieved. This minimizes undesirable product quality changes such as purge, color and texture.

The primary objective of the study was to evaluate the potential synergistic effects of thermal treatment using a saturated steam-based post-process pasteurization system (Townsend) and chemical antimicrobials incorporated into the product formulation to control *Listeria monocytogenes* in Ready-to-Eat (RTE) frankfurters.

METHODOLOGY

A five strain cocktail of *L. monocytogenes* was prepared and "mist" inoculated onto the product surfaces. Products were vacuum packaged and treatments requiring thermal pasteurization were exposed to saturated steam for 5 min 10 s in the Townsend Pasteurizer. The product was subsequently chilled in ice water and the frankfurters were stored at 4°C (ca. 40 °F) for 120 days. *L. monocytogenes* populations were enumerated by plating on selective agar (Modified oxford agar) to determine survival and outgrowth of *L. monocytogenes*.

RESULTS

Application of a post-packaging thermal pasteurization reduced *L. monocytogens* populations by 0.77 log CFU/cm^2 ($p>0.05$). Post-process pasteurization treatment resulted in inhibition of *L. monocytogenes* up to day 15 compared to the non-pasteurized treatments (Table 2). *L. monocytogenes* populations of ca. 6.00 log CFU/cm^2 were attained on control (product that did not contain any antimicrobials) and in product containing lactate/diacetate combination of antimicrobials, where as maximal populations of 3.91 and 2.16 log CFU/cm^2 were observed on product containing citrate/diacetate combination antimicrobials at 1% concentration. Increasing citrate/diacetate combination antimicrobial concentration to 1.3% resulted in greater inhibition of *L. monocytogenes*, with final populations of 2.99 log CFU/cm^2 on day 120.

L. monocytogenes populations of ca. 6.5 log CFU/cm^2 were attained by day 60, in the control product and the product containing lactate/diacetate combination, with minimal increases during subsequent storage up to day 120. Incorporation of lactate/diacetate combination antimicrobial did not result in *L. monocytogenes* inhibition during storage, with similar

populations of ca. 6.8 log CFU/cm^2 attained by day 60, with minimal increases during subsequent refrigerated storage. Incorporation of citrate/diacetate combination antimicrobials with subsequent post-process pasteurization resulted in greater inhibition of *L. monocytogenes* populations, with final populations of 2.44 log CFU/cm^2 attained by day 60, with minimal increases during subsequent storage. Increasing citrate/diacetate combination antimicrobials to 1.3% in the frankfurter formulation resulted in greater inhibition of *L. monocytogenes*, with final populations of 1.78 attained by day 105.

Incorporation of antimicrobials into frankfurter formulation resulted in a recovery of *L. monocytogenes* of 2.37 $\log_{10}$ CFU/cm^2 for citrate/diacetate combination antimicrobial at 1.3% ($p<0.05$, Fig. 2), followed by 1.0% with 3.03 $\log_{10}$ CFU/cm^2 and control and sodium lactate (SL) plus sodium diacetate (SDA) with averages of 6.20 $\log_{10}$ CFU/cm^2 and 6.79 $\log_{10}$ CFU/cm^2, respectively.

Control treatment (no antimicrobial added) and the incorporation of lactate/diacetate combination antimicrobial showed similar *L. monocytogenes* growth during refrigerated storage while citrate/diacetate combination antimicrobial at 1.0 or 1.3% inhibited *L. monocytogenes* growth. In both cases, populations of survival *L. monocyotgenes* reached their maximum levels by day 60 in all the treatments (6.92, 6.34, 2.81, and 2.08 $\log_{10}$ CFU/cm^2 for lactate/diacetate combination antimicrobial, control, citrate/diacetate combination antimicrobial at 1.0% and 1.3%, respectively).

Synergistic effect was not observed for the combination of heat treatment and the addition of antimicrobial in the control of *L. monocytogenes.* However, treatments with citrate/diacetate combination antimicrobial in their formulation inhibited *L. monocytogenes* growth compared to those containing lactate/diacetate combination antimicrobial. Furthermore, no differences were observed between citrate/diacetate combination antimicrobial at 1.0% or 1.3% and whether or not heat treatment was applied.

DISCUSSION

Ready-to-eat meat and poultry products such as frankfurters and deli meats can be contaminated with *L. monocytogenes* by exposure to the environment after the lethality treatment (cooking) during peeling, slicing and/or repackaging. These products are most often consumed without further cooking or reheating, and since the pathogen can grow to high populations under refrigeration temperatures, the risk of foodborne illness is greater from these products.

The results of this study shows that the combination of chemical preservatives generally recognized as safe (GRAS) with a post-lethality thermal pasteurization of artificially inoculated frankfurters (with *L. monocytogenes*) can provide a better bacteriostatic.

In our study, we exposed frankfurters to a surface pasteurization for 5 min and 10 s. Reductions in *L. monocytogenes* populations were minimal in this study as our goal was to evaluate synergistic antimicrobial activity between the antimicrobial agents and the heat, with minimal losses in quality of the products. A synergistic bacteriostatic effect between samples heat treated and antimicrobials was observed in both products during storage.

The *L. monocytogenes* reductions obtained in our study are lower compared to Hardin et al. (2), where the authors evaluated a post-process pasteurization of precooked beef roasts that were previously surface inoculated with *L. monocytogenes*, vacuum packaged and pasteurized at 91ºC or 96ºC for wither 3 or 5 min by submersion heating in circulating hot water. Hardin et al. (2) reported a 4.5 $\log_{10}$ reduction for a 5 min process, while Muriana et al. (3) reported much less reductions in *L. monocytogenes* (ca. 2 log) for most of the RTE deli meats including turkey, ham

and roast beef when processed by submersion heating in a steam-injected water bath and sampling after 48 h. However, there are several differences between the current study and that of Muriana et al., where an inoculum level of 7.0 to 8.0 log CFU/ml was used. The authors suggested that the lower *L. monocytogenes* reduction (2 log) was probably due to the high inoculum level used, as well as collection of purge in cracks or folds in the product surfaces, resulting in protection from the heat. In addition, Muriana et al. reported lower *L. monocytogenes* reductions when the product was exposed to 96.1ºC compared to 90.6 or 93.3ºC. The authors suggested was that at 96.1°C, shrinkage of the packaging film may have squeezed the purge from the chilled interior (4ºC) toward the surface at a faster rate than the rate of heat penetration from the surface, therefore, reducing the overall heating and concomitant microbial reduction. Murphy et al. (90) combined antimicrobials and steam treatments on ready-to-eat franks. These authors reported similar *L. monocytogenes* destruction as Muriana et al (3), on franks exposed to 100ºC for 1.5 s.

An undesirable but inevitable phenomenon that occurs to RTE meat products that are exposed to a heating process is the purge that is generated during the process. In our study, the purge volumes were greater in heat treated hams/frankfurters compared to the product that did not receive a thermal pasteurization. Cygnarowicz-Provost et al. (1) also reported "free water" when beef frankfurters were surface pasteurized by exposure to live steam. However, the authors indicated that equivalent amounts of purge was found on the samples over time regardless of whether the product was heat treated or not.

Several reports have evaluated combinations of salts of organic acids at various concentrations and were shown to be effective in controlling *L. monocytogenes*. In the case of the frankfurters, the combination of citrate/diacetate was more effective in controlling the pathogen at either concentration regardless of the application of a thermal pasteurization. Higher concentration of citrate/diacetate (1.3%) provided a better bacteriostatic effect when combined with heat. In the case of lactate/diacetate, the inhibitory effect was minimal, regardless of the application of the heat treatment. Citrate/diacetate combination antimicrobial at 1.0% showed greater inhibitory effect on the product when the thermal pasteurization was applied, regardless of the temperature of storage. The maximum values for products containing citrate/diacetate combination antimicrobial, whether they were heat treated or not, were below 2.5 $\log_{10}$ CFU/cm^2 during the entire storage period, while product that was not heat treated reached *L. monocytogenes* populations of 2.5 and 3.5 $\log_{10}$ CFU/cm^2.

The combination of salts of organic acids with a thermal pasteurization using saturated steam may help to reduce the potential of recovery of injured *L. monocytogenes*, that in favorable conditions may recover and grow to potentially high populations, thus increasing the risk of listeriosis from RTE meat products.

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